MANNK.021CP1C PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Simard, et al.
Appl. No.	:	10/026,066
Filed	:	December 7, 2001
For	:	EPITOPE SYNCHRONIZATION IN ANTIGEN PRESENTING CELLS
Examiner	:	VanderVegt, Francois P.
Group Art Unit	:	1644

DECLARATION OF ADRIAN ION BOT, PH.D. UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Adrian Bot, declare as follows:

1. I am currently a Senior Director of Research and Development at MannKind Corporation. Prior to joining MannKind in 2002, I was a Principal Scientist and Director of Immunology at Alliance Pharmaceutical Corp. in San Diego, CA (1998-2002) as well as a Guest Scientist at the Scripps Research Institute in La Jolla (1998-1999). My technical background is in theoretical and experimental immunology, autoimmune disorders, viral immunity and cancer immunotherapy. My credentials include an M.D. from the University of Medicine and Pharmacy in Timisoara – Romania (1993), and a Ph.D. in Immunology / Microbiology from Mount Sinai School of Medicine in New York (1998). I have authored or co-authored more than 45 peer-reviewed research articles, reviews, book chapters and a monograph, in basic and applied immunology, and am an inventor on patents and patent applications dealing with DNA vaccines, microparticle-based technologies, immune therapeutic strategies and compositions. I serve on the Scientific Advisory Board of the Cancer Vaccine Consortium, was for several years an Associate Editor of the *Journal of Immunology* (2001-2005), and am now the Editor in Chief of the *International Reviews of Immunology*.

2. I have been asked to give my opinion as to certain questions as an expert in antigen processing and as a person familiar with the level of skill in the art. It is my understanding that these questions are related to factual issues that have arisen in the above-referenced patent application. In this Declaration, I will state each question in a numbered paragraph and will provide my opinion relating to that question in one or more numbered and lettered paragraphs.

3. **FIRST QUESTION**: Is the group of epitopes that the applicants refer to as "housekeeping epitopes" a meaningful class of epitopes that clearly encompasses some epitopes and excludes other epitopes?

3A. As defined by the applicants, housekeeping epitopes are members of a group of epitopes that share certain common characteristics that in combination are not shared with non-housekeeping epitopes.

3B. One key feature that housekeeping epitopes all share is that they are polypeptide fragments of larger proteins and are formed via activity of the housekeeping proteasome that is predominantly active only in certain cell types under certain conditions. Specifically, the housekeeping proteasome is predominantly active in peripheral cells, including neoplastic or chronically infected cells, so long as such peripheral cells are not undergoing interferon-induced gene expression. Housekeeping proteasomes are not predominantly active in pAPCs.

3C. Another key feature that housekeeping epitopes all share is that each has an affinity for at least one allele product of class I major histocompatibility complex (MHC).

3D. Another key feature that housekeeping epitopes all share is that they are displayed on the surface of cells in which the housekeeping proteasome is predominantly active.

3E. The application describes the desired cell type for producing a housekeeping epitope which, in and of itself, provides a meaningful and useful definition of a housekeeping epitope. This description correlates with the defining characteristics of the genus. Figure 1, attached hereto, is a schematic representation based upon the disclosure of the application, showing the ease and clarity with which such a cell type can be recognized.

3F. It is my opinion as an expert in antigen processing that these common features do indeed define a class of epitopes that is a subset of all epitopes and that can be referred to as housekeeping epitopes, and that the features listed in paragraphs 3B, 3C, and 3D are only all

present in housekeeping epitopes. Members of this class are therefore clearly distinguishable from non-members of this class.

3G. The housekeeping epitopes generated from any given protein constitute collectively a discrete and reproducible population of epitopes. The immune epitopes generated from the same given protein constitute collectively a different, discrete and reproducible population of epitopes. While these populations will share common members, the populations themselves are clearly distinguishable. Those members of the housekeeping epitope population all share the common features discussed in paragraphs 3B, 3C, and 3D. In contrast not all the members of the immune epitope population share these common features. See Figure 2.

3H. Recognition of those individual epitopes that are housekeeping epitopes is set forth in the patent application in such a way that it is clearly distinguishable whether a given epitope is a housekeeping epitope or is not a housekeeping epitope.

4. **SECOND QUESTION**: What are the defining features of the genus of housekeeping epitopes?

4A. The defining features of the genus of housekeeping epitopes are set forth above in subparagraphs 3B, 3C, and 3D. Housekeeping epitopes all (1) are protein fragments formed via activity of the housekeeping proteasome; (2) have an affinity for class I MHC; and (3) are displayed on the surface of cells in which the housekeeping proteasome is predominantly active.

4B. It is my opinion that these characteristics of the genus of housekeeping epitopes are completely diagnostic of this class of epitopes. I am unaware of the existence of any other factors that would be necessary to further distinguish between housekeeping epitopes and non-housekeeping epitopes.

5. **THIRD QUESTION**: As a substitute for, or in addition to, the defining features of housekeeping epitopes discussed above, what could be generalized by having a large number of examples of members of this genus?

5A. In my opinion, numerous specific examples of housekeeping epitopes, providing the sequences of individual epitopes, would not assist further in defining the genus of housekeeping epitopes or in distinguishing between members of this genus and non-members.

5B. Information is embodied within the structure of housekeeping epitopes, including the epitope sequence, the secondary structure, and the physical/chemical characteristics of such

epitopes. Such information defines housekeeping epitopes, and is based upon the common features of all housekeeping epitopes as defined above. Thus, there are no amino acid sequence motifs that could substitute for or be generalized to encompass all housekeeping epitopes and to exclude all non-housekeeping epitopes.

5C. The statements recited in subparagraphs 5A and 5B are due in part to the diversity of class I MHC receptors and are also due in part to the complex relationships between protein sequence, secondary and tertiary structure, and proteasomal cleavage site specificities. Thus, the variety of different antigen sequences, the complexity of their interaction with the proteasome, and the diversity of MHC class I binding requirements, makes it impossible to give a sequence common to all epitopes.

5D. It is incorrect and simplistic to expect that all relevant structure or characterization information lies only in the primary amino acid sequence of a class of epitopes. In my opinion, quite the contrary is true. There are common characteristic features shared by all housekeeping epitopes (most readily appreciated by the biological processes), and these features define the members of this genus.

5E. It is also incorrect and simplistic to dismiss these common features as failing to describe structural properties shared among the class of housekeeping epitopes. Each of the common features as set forth in 3B, 3C, and 3D is highly correlated with structure. For these epitopes, function is dependent upon structure, and function is a proxy for the underlying structure from which it is so specifically formed from the antigenic protein.

5F. It is also incorrect and simplistic to dismiss these common features as defining the source of an epitope, but not the epitope itself. An epitope is a housekeeping epitope by virtue of structure-specific considerations. However, the complex structural interactions of proteasomes and proteins do not permit formulaic generalization of the underlying structural requirements. The structural requirements that determine proteasome and protein interactions, and epitope-MHC interactions, are so complex that deriving a formulaic or motif-based generalization of the genus of housekeeping epitopes would require an enormous expenditure of effort and resources. Further, one would not need to undertake such an expenditure of resources when members of the genus of housekeeping epitopes can be recognized easily and clearly based upon the criteria provided by the applicants, as described above.

5G. Employing simple functional criteria as proxies for highly complex structural characteristics is common and widely accepted in biology. There are numerous examples of important types of biomolecules and systems whose classification, recognition, and practical uses are based upon this approach. Particularly instructive is the case of antibodies. The general structure of antibodies and their various types and subtypes are known in detail at the atomic level. However the structural details distinguishing an antibody of one specificity from another are generally not determined. To the contrary, for practical uses antibodies are defined and grouped functionally according to the antigen(s) recognized or the function they perform, and not by the sequence of the CDRs (complementarity determining regions). Indeed CDR sequence similarity is a poor predictor of common specificity and common specificity can arise from distinctly different sequences.

5H. Some additional examples in support of the facts recited in subparagraph 5G are: enzymes, signal sequences, receptors, and binding proteins. Each of these acts by binding to or interacting with a substrate or binding partner based upon structural characteristics, although the precise structures responsible for such interactions are usually not fully characterized. Each of the foregoing examples, however, is classified, recognized, and used based upon its functional characteristics without requiring a complete determination and description of the underlying structure or sequence.

5I. For all of the foregoing reasons, it is my opinion that the structure of the class of housekeeping epitopes is best represented and generalized on the basis of the common characteristics of this genus, and that numerous exemplary epitope sequences would not serve to further define the genus. In my opinion and as explained above, there is not a better possible description of a generalizable structure of the members of this genus than the description provided by the applicants in their patent application. Indeed, as with antibodies and other biomolecules as mentioned in subparagraph 5H, it is the biological outcome (cleavage by housekeeping proteasome followed by binding to class I MHC) rather than primary structure that are ultimately more informative for recognizing the member of the genus.

6. In summary of Paragraphs 3, 4, and 5 and their subparagraphs, it is my expert opinion that the class of housekeeping epitopes as defined by the applicants is indeed a true and valid genus with clear members and non-members, and that the description by the applicants of

this genus and its structural characteristics is complete and is superior to a description based upon numerous examples of epitope sequences. For the foregoing reasons, it is my expert opinion that the applicants have clearly and sufficiently defined the structural differences between housekeeping epitopes and non-housekeeping epitopes.

7. **FOURTH QUESTION**: Would a person of ordinary skill in the art of immunology recognize the existence of housekeeping epitopes as a genus, based upon the applicants' description of the invention?

7A. A person of ordinary skill in the art of immunology, upon reading the applicants' description of the class of housekeeping epitopes, would recognize that there is a combination of key diagnostic features that all housekeeping epitopes share. A person of skill in the art would further recognize that non-housekeeping epitopes do not share this combination of features.

7B. A person of ordinary skill in the art would recognize that the key diagnostic features of housekeeping epitopes are those that have been discussed in the foregoing paragraphs and subparagraphs, particularly in the subparagraphs of Paragraph 3, above.

7C. A person of ordinary skill in the art would readily be able to make use of the recited diagnostic features in distinguishing between members and non-members of the genus of housekeeping epitopes.

7D. A person of ordinary skill therefore would recognize the existence of this genus and would appreciate what the genus includes and what it excludes.

8. **FIFTH QUESTION**: From the disclosure, would a person of ordinary skill in the art conclude that the applicants were in possession of the entire genus of housekeeping epitopes?

8A. A person of ordinary skill in the art, reading the applicants' description, would recognize that the applicants have described the key features needed to define housekeeping epitopes as a genus.

8B. A person of ordinary skill in the art would appreciate that, for the applicants to have described the genus of housekeeping epitopes according to its distinguishing features, and appropriate methods to recognize the genus, the applicants must have conceptually understood and generalized the entire genus according to those features.

8C. A person of ordinary skill would therefore conclude that the applicants were in possession of the entire genus at the time the description was written.

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9. **SIXTH QUESTION**: What would a person of ordinary skill in the art understand, as to either of the foregoing questions, if the disclosure instead provided a large number of examples of housekeeping epitopes?

9A. A large number of exemplary sequences, without a description of the common features that together define the class of housekeeping epitopes, would not assist a person of ordinary skill in the art to recognize that this class exists as a discrete genus, nor would it provide evidence to a person of ordinary skill that the applicants were in possession of such a genus. The common features described by Applicants are sufficient to show possession of the entire genus.

9B. This is because it would not be possible for a person of ordinary skill in the art to detect, from a large number of exemplary sequences, the common features and structure of this class of epitopes. In fact, the disclosure of even a single housekeeping epitope by Applicants, coupled with the disclosure of all of the relevant identifying characteristics as recited herein, is sufficient to exemplify and show possession of the genus of housekeeping epitopes.

9C. A large number of exemplary sequences would therefore be far inferior to the applicants' description in permitting a person of ordinary skill in the art to recognize the existence and characteristics of this genus, or to recognize that the applicants were in possession of this genus.

10. In summary of paragraphs 7, 8, and 9, and their subparagraphs, it is my opinion that a person of ordinary skill in the art, reading the disclosure of the above-referenced patent application, would fully recognize the existence and essential characteristics of the genus of housekeeping epitopes, and would recognize that the applicants were in possession of the entire genus at the time the application was filed.

11. **SEVENTH QUESTION**: Having defined the existence and essential characteristics of the genus of housekeeping epitopes, is the group of T-cells that the applicants refer to as T-cells that "express a T-cell receptor specific for an MHC-peptide complex comprising a first housekeeping epitope" a meaningful class of T-cells that clearly encompasses some T-cells and excludes other T-cells?

11A. As defined by the applicants, and discussed in paragraphs 3B, 3C, and 3D, housekeeping epitopes are members of a group of epitopes that share certain common characteristics that in combination are not shared with non-housekeeping epitopes. As discussed

in paragraph 5E, each of these common features is highly correlated with structure. For these epitopes, function is a proxy for the underlying structure, and function and structure are inextricably linked.

11B. It is well known in the art that for a T-cell receptor to bind to an MHC-peptide complex, the T-cell receptor must have a structure that permits binding the MHC-peptide complex. That is, a T-cell binds an MHC-peptide complex based upon the recognition of a specific peptide-MHC complex by a unique T-cell receptor expressed by the T-cell. Thus, once a specific MHC-peptide complex has been defined, the receptor that binds that target has also been defined.

11C. As discussed in paragraph 7C, a person of ordinary skill in the art would readily be able to make use of the recited diagnostic features in distinguishing between members and non-members of the genus of housekeeping epitopes. Because a particular T-cell receptor is specific for a particular target, a person of ordinary skill in the art would be able to distinguish the T-cells of the claimed compositions from other T-cells based on the description of housekeeping epitopes provided by the applicants.

12. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: 03.27.06 By: Adrian Bot

Adrian Bot

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Figure 1. Illustration of the aggregate, required characteristics of cells (in red) whose housekeeping proteasome would convert a protein (blue) into housekeeping epitopes (blue).

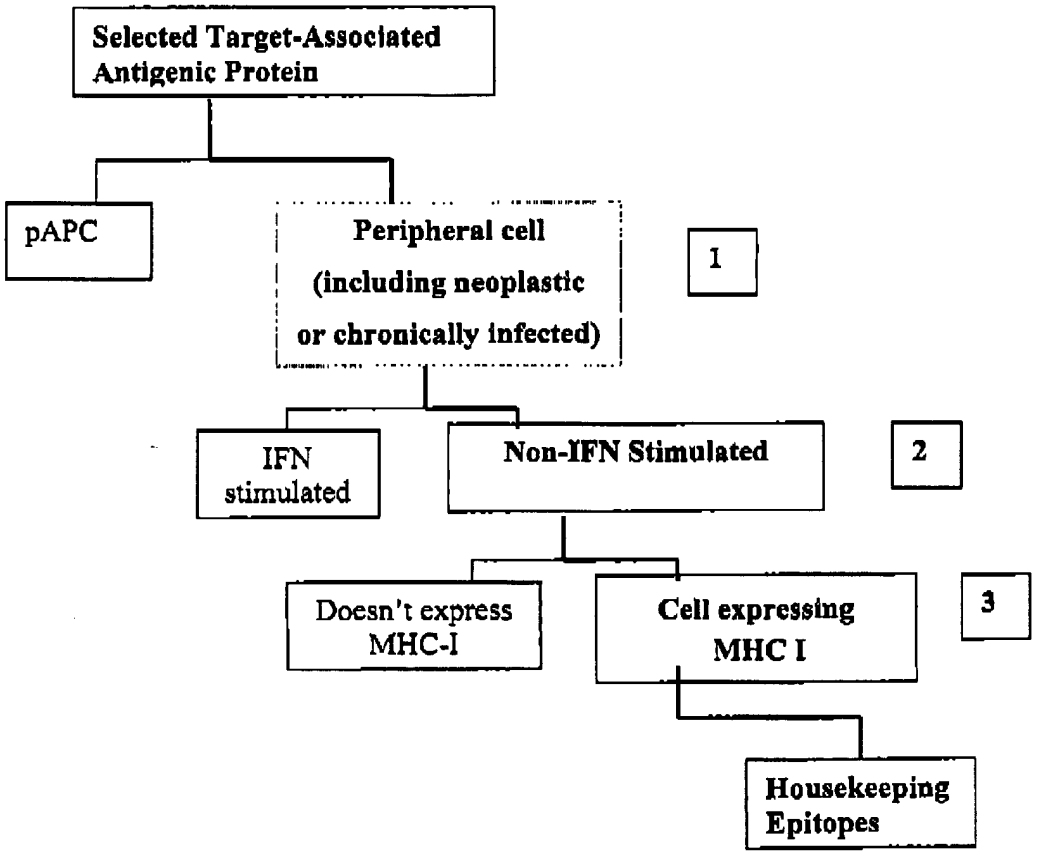

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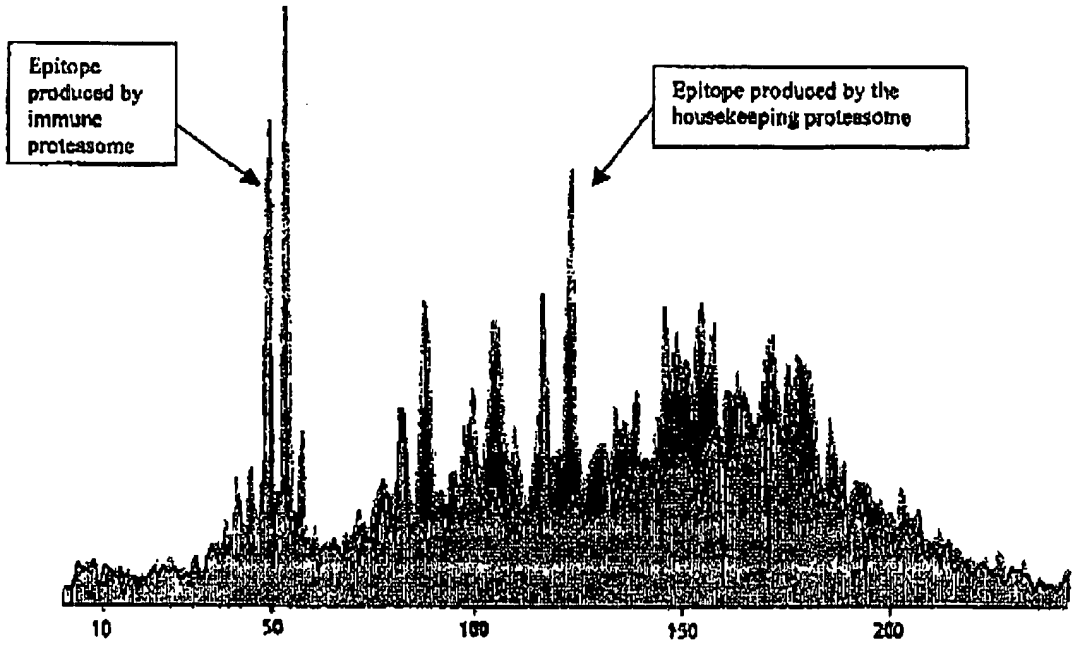
Figure 2 illustrates an HPLC overlay of a protein metabolized by a housekeeping proteasome and under similar conditions by an immunoproteasome stimulated by interferon. The products from the housekeeping proteasome are in blue; the products from the immunoproteasome are overlaid in green.

Figure 1. HPLC chromatograms (superimposed) of epitopes produced by either immune proteasome in cells stimulated by IFN or housekeeping proteasome in cells not stimulated by IFN. This figure is taken from one of 4 replicate experiments demonstrating reproducibility.



Adapted from Maksymowych, et al, Infection and Immunity.